GALLS ON EUCALYPTUS TREES.

A NEW TYPE OF ASSOCIATION BETWEEN FLIES AND NEMATODES.

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(Plates vi–vii; thirty-one Text-figures.)

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Introduction.

The flower buds of many *Eucalyptus* trees, particularly *Eucalyptus camaldulensis* Dehn. and *E. hemiphloia* F.v.M., are galled during certain seasons to such an extent that very few of them develop into normal flowers. Whole branches may break under the weight of galls. The trees named, and others, are the source from which much of the Australian honey is harvested, so galling restricts the honey output.

Galls of similar type were found affecting the flower buds of *Eucalyptus maculata* Hook., a valuable timber tree, so that the production of seeds was reduced. Morgan (1933) discovered that the organisms causing the galls on these trees were small nematodes and Agromyzid flies of the genus *Fergusonina*. Working independently, the writer had meanwhile been studying the galls on the flower buds of *E. macrorrhyncha* F.v.M. and found that they were caused by flies of the genus *Fergusonina* working in symbiotic association with small nematodes which were found to be closely allied to certain small plant-parasitic genera. Further work revealed that galls caused by the flies and nematodes, always associated, were common in all parts of Australia on many *Eucalyptus* trees. Galls were present on leaf buds, axil buds, stem-tips, and flower buds. In the photograph (Pl. vi, fig. 1), the difference between galled and ungalled flower-buds is clearly discernible, while the photograph of *E. macrorrhyncha* (Pl. vi, fig. 2) shows to what an extent galling may affect trees.

A review of literature on Australian galls disclosed that Rübsaamen (1894) had recorded dipterous larvae in flower-bud galls from Queensland. The species of tree is not stated, but the illustration resembles flower-bud galls found on *E. melanophloia* F.v.M. Cambage (1918), writing on the flora of the Federal Capital Territory, records finding galls caused by the larvae of Agromyzid flies on flower buds of *E. dealbata* (probably this was *E. Blakelyi* Maiden), the galls being about 14 mm. in diameter, whereas the normal flower bud was only about 2 mm. wide. Beuhne (1923), in the "Honey Flora of Victoria", refers to flower-bud galls on *E. camaldulensis*, but does not mention the cause.

Below are given the results of some five years' study of the gall-producing insects, the associated nematodes, and of the galls they produce.

Life History of Fly and Nematode.

There are many species of *Fergusonina* flies which attack *Eucalyptus* trees, and all are associated in the galls with nematodes. The fly which causes the galls on *E. macrorrhyncha* was studied most intensively, so the life history of that fly is presented here.

Adult flies emerge from the galls in summer, and the females, after mating, proceed to lay eggs in the young flower-buds which are appearing at that time. With each egg, any number of larval nematodes from one to fifty is passed into the cavity between the operculum and the floor of the inside of the bud. Many eggs may be laid in the same bud by a single fly or by several flies, and as many as 74 eggs and 227 nematode larvae have been found in a single bud.

Embryonic development within the egg of the fly proceeds during the next six weeks (eggs which were laid on 15th December hatched on 1st February). During that period the larval nematodes feed vigorously on the primordia of the stamens and cause a rapid proliferation of cells which form irregular masses inside the galled bud.

On hatching, the fly larvae make their way between two contiguous masses of cells and tear out small crypts in which to lie. The larval nematodes join them in their several crypts and develop rapidly to the adult stage. The nematodes of that generation are all parthenogenetic females which lay eggs in the gall cavity alongside the fly larva, with which they lie in contact. The fly larva passes through three instars, all in the crypt inside the galled bud, obtaining its food from the plant cells surrounding it. During the first and second instars it feeds on the gelatinous cell-sap, some of which oozes from the cells after they have been punctured by the stylets of the nematodes. The third instar larva tears down the walls of the cavity in which it lies and feeds on the ruptured cells.

The nematodes breed parthenogenetically in the cavity during the larval life of the fly without harming it in any way, males appear in numbers in the autumn and winter, and when the female fly larva is about to pupate, two fertilized female nematodes enter its body cavity, probably through the skin. There, during the pupal period of the fly, the female nematodes change from the free-living form to a much enlarged parasitic form which has no stylet or gut, the whole of its internal space being filled by a much enlarged ovary. Male flies are never parasitized in this way by the nematodes, female flies invariably so. By the time the female fly emerges adult the parasitic nematodes are discharging large numbers of segmenting eggs inside its body cavity. On hatching, larval nematodes make their way to the ovary, penetrate into the oviduct, and there await the passage of an egg down the chitinous ovipositor, whence they accompany it into the young flower-bud to start the cycle anew.

This life history can be taken in its broad outlines as typical for the whole series of flies. The time of year when adults emerge and the point of the tree attacked vary, but young growing tissue is always selected by the flies for oviposition, and the nematode larvae which are always deposited with the eggs of the fly are active before the eggs hatch.

Methods.

Larvae of flies.—Larvae of different ages were dissected alive from the galls and the natural outlines drawn with the camera lucida. For detailed study of skin structures and mouth parts, larvae were boiled in caustic potash, then the

skins were washed, cleared in glacial acetic acid, stained with acid fuchsin and mounted in Canada balsam.

The adults.—Female flies bred out in the laboratory were dissected daily in saline to study the development of the ovaries and of the parasitic nematodes. Others were placed in organdi sleeves enclosing young flower-buds so that oviposition could be observed and buds labelled with the date on which eggs had been laid in them.

The nematodes.—Minute studies of the internal organization of the free-living nematodes were made on fresh material just immobilized by heat. A small cell on a glass slide was found to answer well for this purpose. Parasitic females had to be studied in normal saline; in water they rapidly swell up and burst. Semi-permanent mounts were made with glycerine.

The galls.—Fresh galled buds were collected and studied throughout the years and much information was obtained in this way. Some sections of the galls were kept for permanent record, but no wholly satisfactory technique was discovered to fix and preserve equally well the vegetable tissues, the nematodes and the fly larvae. Alcoholic Bouin penetrated rapidly in vacuo and the material stained, dehydrated, cleared, and mounted in hard paraffin gave some reasonably good sections.

Elements in the Association.

I. *The Fly.*

The adult flies bred out from galls during the investigation have been described by my colleague, Mr. A. L. Tonnoir (1937), who has revised the genus *Fergusonina*. Malloch named the genus for the noted Australian dipterist, Dr. Ferguson, and classified it in the family Agromyzidae; the genus is somewhat aberrant, however, and Tonnoir has decided to place it in a subfamily by itself. All the flies are small, with a wing span ranging from 5 mm. to about 7 mm., and are of a mottled yellow and black colour. The females have strong ovipositors similar to those of the Trypetidae (Pl. vii, fig. 1). They were found to be rather weak fliers and did not readily take wing.

In warm weather mating takes place about 48 hours after emergence, but is delayed in cold weather; the adult flies live in the summer months from about 6 to 20 days only. They have not been seen feeding in the open, but in captivity they suck up water or sugar syrup readily. Egg-laying generally takes place during the hours from 10 a.m. to 2 p.m. The females appear to lay equally in shade or in the sun during warm weather, but during cool weather the ovipositing females congregate on the sunny side of the trees. Owing to this habit it was observed that, when the weather was colder than usual during the period when flies were most common, the northern side of the trees was more heavily galled than the southern; when temperatures were fairly high, however, most of the buds on the tree were galled. When the galls mature, and just for a few weeks afterwards, the flies are extremely common, and the fact that they have not been taken and described more frequently than has been the case in the past is probably due to four factors: (1) Their small size; (2) their short life as adults; (3) their habit of clinging closely to the branches and not flying readily; (4) that little attention has been given by collectors in Australia to the small diptera.

Emergence of Adult Flies from Galls.

A considerable number of different methods for the emergence of the adult flies from the galls have been developed by the different species of flies.

F. nicholsoni, which has been discussed earlier, emerges when the operculum of the galled flower-bud lifts. When the flower-bud galls of *E. Blakelyi* are fully mature the inner portions dry up and break up into a powder, through which the adult flies (*F. tillyardi*) escape to the exterior; the galls frequently drop to the ground at maturity and the flies escape from them there. The larvae of *F. curriei* living in the community leaf-bud galls of *E. macrorrhyncha* burrow when full fed to a point just under the skin of the gall and there pupate; the adult fly can then push its way readily to the exterior. The larva of *F. greavesi* cuts a round hole in the wall of the gall chamber before it pupates, leaving only the epidermis unbroken; through this the fly escapes at emergence. This last method of emergence is commonly used by the various larvae inhabiting leaf and stem-tip galls.

The puparia which inhabit the leaf, axil bud, and stem-tip galls are all found attached to the wall of the galls by a transparent elastic jelly fixed to the anal end. This gelatinous material, which is voided by the full-fed larva just before pupation, holds the puparium in position at the anal end while the adult fly bursts its way through the anterior end. This substance is absent from most of the puparia found in the flower-bud galls.

Description of immature stages of Fergusonina nicholsoni *Tonn.*

This species from flower-bud galls on *E. macrorrhyncha* is taken as a type to illustrate the stages in the life history of flies of the genus *Fergusonina*.

Egg.—The egg is a spindle-shaped, transparent, glistening body. It tapers to a sharp point at one end and to a more rounded tip covered by a cap at the micropylar end (Fig. 1). Length of egg, 0·33 to 0·4 mm.; width of broadest part, 0·1 mm.

First instar larva (Fig. 2).—At hatching the larva is shorter than the egg from which it hatches. The mouth parts are 0·04 mm. long at this stage. No signs of spiracles or tracheae have been observed in this instar. Rows of papillae are clearly distinguishable along the line of junction of segments on the dorsal surface. Length, 0·23 mm.; width, 0·07 mm.

Second instar larva (Fig. 3).—This larva is immobile and lives in a small close-fitting cavity surrounded by nematodes bathed in a mucilaginous fluid apparently exuded by the cells lining the gall cavity. Its skin is extremely delicate and transparent, all the internal organs are easily distinguishable, and the papillae marking the junction of the segments of the dorsum are very pronounced. The mouth parts are minute in proportion to the bulk of the larva and do not appear to be used for tearing, the larva imbibing the fluid in which it lies. The actual size of the mouth parts is less than that of the first instar, a contradiction to the normal rule, the length being only 0·024 mm. No sign of a tracheal system can be distinguished in this instar. Length, 0·9 mm.; width, 0·7 mm.

Third instar larva (Fig. 4).—From the delicate 2nd instar larva the 3rd instar emerges as a vigorous, tough-skinned larva, with strong mouth-parts and heavily-chitinized spiracles opening into a well-defined tracheal system. The respiratory system is amphipneustic. The mouth parts are 0·133 mm. long. On the dorsal surface is a strongly-chitinized dark-brown plate not present on the first and second instars which, for the sake of convenience, one may call the "dorsal shield", extending from the first thoracic segment over the first and second abdominal segments. The general shape of the larva is sub-ovate, but

many other species of *Fergusonina* have pyriform larvae. Length, 1·3 mm.; width, 0·9 mm.

Structure of spiracles and mouth parts of Fergusonina *larvae.*

The *spiracles* are fairly similar in shape throughout the series of larvae discovered so far. The three slits of the spiracles are raised on protruding lips resembling the corolla of a flower (Fig. 22). The anterior slit of the anterior spiracles is nearly as big as the other two slits combined. Small elliptical perforations through the slits allow air to enter the felt chamber, which is walled with brown chitin, and this in turn leads into a large trachea. The posterior spiracles are remarkably similar to the anterior, both in size and shape.

Figure 22 illustrates, by camera lucida drawings, the spiracle of 3rd instar larvae from leaf galls on *E. maculosa* and the spiracle of 3rd instar larvae of *F. tillyardi*. Such differences as can be observed between these two represent the amount of difference to be seen between the most dissimilar pair of the series of larvae.

The larvae from the flower-bud galls of *E. pauciflora* have smaller spiracles than the others, and the chitin forming them is not so deeply pigmented, but otherwise the structures are very similar.

The *mouth parts* (bucco-pharyngeal apparatus) are fairly regular in shape throughout the series. Two sets are illustrated in Figures 23 and 24. Figure 23 is a drawing of the mouth parts of *F. tillyardi* Tonn., and Figures 24 and 25 of those of *F. eucalypti* Mall. These two sets have been chosen because they show as great a difference between them as can be found between any two species of the genus.

The Puparium.

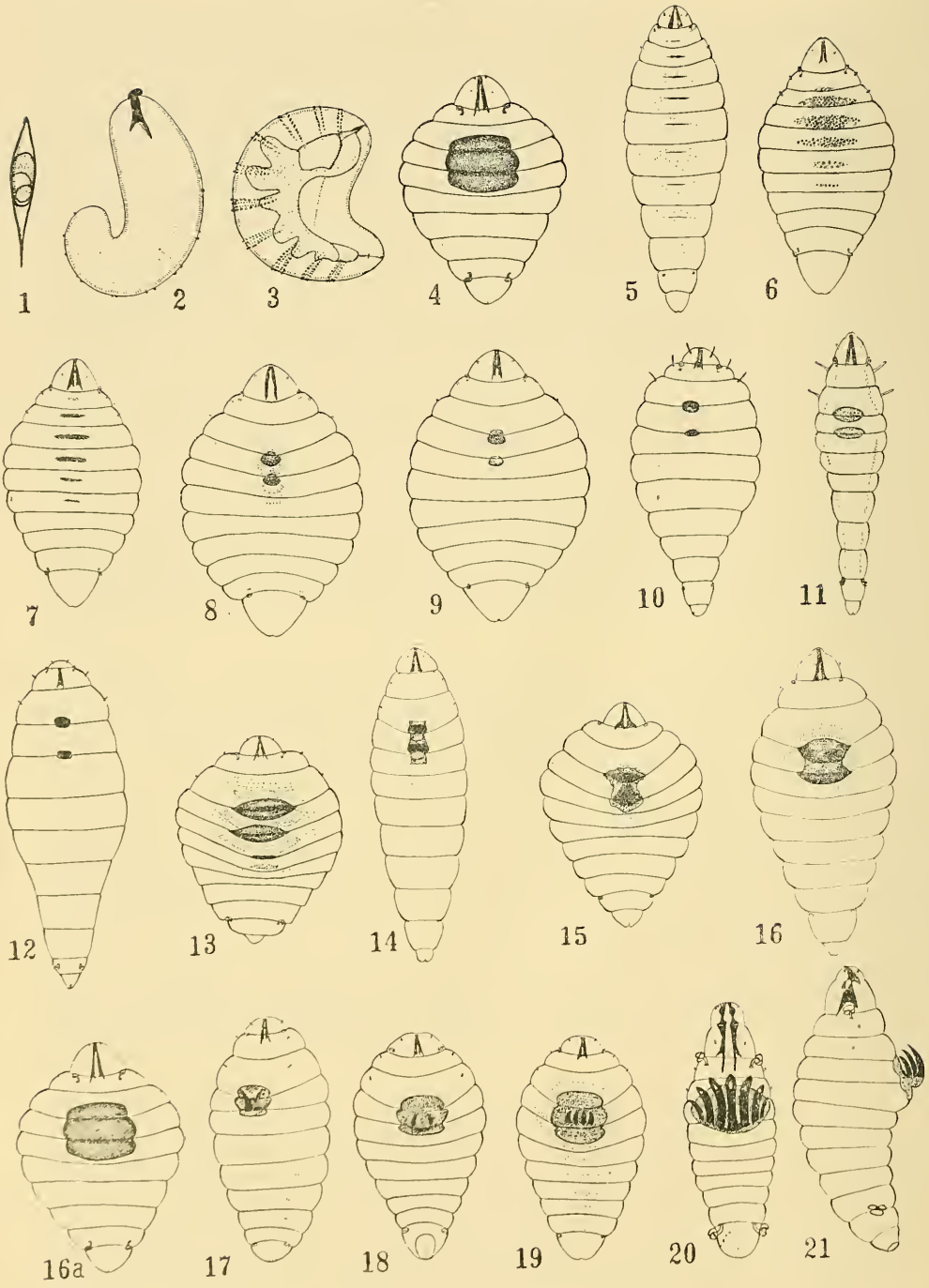
The puparium lies in the eaten-out cavity of the gall until the emergence of the adult fly. In form it is the typical barrel-shaped dipterous puparium of the Cyclorrhapha, with no distinctive characters, and varies in colour from light straw on pupation to dark brown just before emergence of the fly.

Description of 3rd Instar larvae of Fergusonina *species with special reference to the dorsal "shield".*

The mouth parts of spiracles did not appear to have diagnostic value, but the dorsal shield on the various larvae proved to be a useful diagnostic feature. The sequence in which the third instar larvae are described indicates the possible direction of the evolution of this organ.

The dorsal shield, which is considered to be the most primitive of those discovered, consists merely of scattered spots of chitin on the terga of the 2nd and 3rd thoracic and 1st, 2nd, 3rd, 4th, 5th, and 6th abdominal segments. A series of shields of increasing complexity then follows, culminating in one which consists of a black plate from which a strong rake-like organ, carrying from 5 to 7 teeth, projects outwards.

1. *Fergusonina* sp. 1 (Fig. 5).—From stem-tip gall on *E. pauciflora*, Mount Kosciusko, N.S.W., June, 1935; galls collected by M. J. Mackerras. This larva carries small spots of chitin over the greater part of the dorsum. Long narrow patches, composed of closely-set spots of chitin, are present on the 2nd and 3rd thoracic and the 1st to the 6th abdominal segments. These patches are on the most projecting parts of the convex segments, so that they come into contact frequently with the walls of the gall in which the larvae live. Paired papillae are present on each of the three thoracic segments, two pairs on the first and one pair each on the other two segments. The spiracles are prominent, and open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into the amphipneustic respiratory system. The adult has not yet been described. Length of full-grown larva, 5 mm. approx.; width of full-grown larva, 1·6 mm. approx.

2. *Fergusonina* sp. 2 (Fig. 6).—From stem-tip on *E. macrorrhyncha*, Black Mountain, F.C.T., Sept., 1934; collected by the author. This 3rd instar larva carried a dorsal shield formed of scattered spots of chitin on the dorsum. There are chitinous cones on the 2nd and 3rd thoracic and on the 1st, 2nd, 3rd and 4th abdominal segments, each cone being surrounded at its base by smaller spots of chitin. Paired papillae are present, as in the species just described, and the spiracles are very similar. Length of full-grown larva, 1·7 mm. approx.; width of full-grown larva, 1·0 mm. approx.

3. *Fergusonina curriei* Tonn. (Fig. 7).—From leaf galls on *E. macrorrhyncha* (see photo, Pl. vi, fig. 5), Canberra, F.C.T., Oct., 1933; collected by the author. In this larva the dorsal shield is present on the same six tergites as in the former species, and sometimes extends to the fifth abdominal segment. The chitinized spots are somewhat irregular and coalesce on each segment to form a more or less coherent plate; this distinguishes it from the species previously described. The papillae on the thoracic segments are somewhat smaller than those of the preceding species, and the larva is much larger. Length of full-grown larva, 3·7 mm.; width of full-grown larva, 2·3 mm.

4. *Fergusonina* sp. 3 (Fig. 8).—From leaf galls on *E. sideroxylon*, *E. maculosa*, *E. melliodora* and *E. macrorrhyncha* in Victoria and in New South Wales, 1933–34. In this species the dorsal shield represents a further step in specialization. Coherent chitinized patches are found in two places, the anterior at the junction of the third thoracic segment with the first abdominal, and the posterior, at the junction between the second and third abdominal segments. Chitinous spots are scattered round both areas. The adult of this species has not yet been bred out and, as the larva is not very different from others which follow, it is not considered desirable to name it at present. Length of full-grown larva, 2 mm.; width of full-grown larva, 1·4 mm.

5. *Fergusonina* sp. 4 (Fig. 9).—From leaf galls on *E. maculosa*, Black Mountain, Canberra, F.C.T., July, 1934; collected by the author. This larva is very like the preceding, but has a regular slight difference in the shape of the dorsal plates, and has fewer spots around the shield. It may be a variety of the preceding species, but the adults have yet to be bred to give evidence on this point. Length of full-grown larva, 2·3 mm.; width of full-grown larva, 1·4 mm.

6. *Fergusonina evansi* Tonn. (Fig. 10).—From leaf galls on an unidentified *Eucalyptus* tree in Adelaide, South Australia, August, 1933; collected by J. W. Evans; and from leaf galls on *E. melliodora* at Canberra, October, 1934, by the author. The dorsal shield differs somewhat in shape from the foregoing species, and the papillae on the thoracic segments are elongated, apparently in adaptation to the comparatively large gall-cavity in which the larva lives. Length of full-grown larva, 2·7 mm.; width of full-grown larva, 1·5 mm.

Text-figures 1-21.

1-4.—*Fergusonina nicholsoni* Tonn. 1, egg, × 50; 2, first instar larva, × 50; 3, second instar larva, × 45; 4, third instar larva, × 25.

5-21.—Third instar larvae of *Fergusonina* species.

5, *Fergusonina* sp. 1, × 8; 6, *F.* sp. 2, × 18; 7, *F. curriei* Tonn., × 8; 8, *F.* sp. 4, × 18; 9, *F.* sp. 5, × 18; 10, *F. evansi* Tonn., × 12; 11, *F. carteri* Tonn. × 28; 12, *F. greavesi*, n. sp., × 8; 13, *F. eucalypti* Mall., × 12; 14, *F.* sp. 10, × 8; 15, *F.* sp. 11, × 10; 16, *F.* sp. 12, × 16; 17, *F. newmani* Tonn., × 16; 18, *F. brimblecombei* Tonn., × 22; 19, *F. lockharti* Tonn., × 12; 20, *F. tillyardi* Tonn., × 22; 21, *F. tillyardi* Tonn., × 14.

Fig. 16*a* is a repetition of Fig. 4 in its place in the series.

7. *Fergusonina carteri* Tonn. (Fig. 11).—From leaf galls on *E. Stuartiana* F.v.M., Canberra, F.C.T., November, 1933; collected by the author. The dorsal plates are clearly defined in the larvae of this species and not surrounded by spots of chitin. The papillae are very large, the larvae living in gall cavities which are large relative to the size of the larvae. Length of full-grown larva, 1·2 mm.; width of full-grown larva, 0·4 mm.

8. Fergusonina greavesi, n. sp. (Fig. 12).—From stem-tip galls on *E. polyanthemos* Schau., Black Mountain, Canberra, July, 1934; collected by the author. The 3rd instar larva of this species is comparatively large, found rather rarely in stem-tip galls of *E. polyanthemos* Schau. The adult fly has not been reared, but the larva is so distinct in size, and in the character of the dorsal shield, that it is felt that the species may be described from the larva without danger of creating difficulties for later workers. It is anticipated, moreover, that the adult flies of this species will be bred out from the galls by the author at some later date, and can then be described in association with the larvae, so that no confusion of identification may arise. The full-grown larva is smooth, clearly segmented, yellowish, with four chitinous plates, in two pairs, on the dorsal surface. One pair is in contact at the junction of 3rd thoracic segment with the 1st abdominal, the other pair is in contact at junction of 1st with the 2nd abdominal segments.

Size of plates approximately: (1) Plate on 3rd thoracic segment, width 0·25 mm., depth (i.e., anterior to posterior margin) 0·12 mm.; (2) Anterior plate on 1st abdominal segment, width 0·2 mm., depth 0·033 mm.; (3) Posterior plate on 1st abdominal segment, width 0·2 mm., depth 0·075 mm.; (4) Anterior plate on 2nd abdominal segment, width 0·25 mm., depth 0·06 mm. Length of full-grown larva, 4·3 mm.; width at widest part, 1·8 mm.

Holotype and paratype on slides in museum of Division of Economic Entomology, C.S.I.R., Canberra, F.C.T., Australia.

9. *Fergusonina eucalypti* Mall. (Fig. 13).—From flower-bud galls of *E. maculata* Hook., Bateman's Bay, N.S.W., September, 1930; collected by W. L. Morgan. In the 3rd instar larva of this species the chitinous dorsal shield is made up of four plates, relatively much larger than those of the foregoing species, and a fifth smaller plate situated at the posterior margin of the 2nd abdominal segment. Rows of chitinized spots surround the plates extending on to the 3rd and 4th abdominal segments. The thoracic tubercles are short, the larvae inhabiting gall cavities into which they fit fairly tightly. Length of full-grown larva, 3 mm.; width of full-grown larva, 1·8 mm.

10. *Fergusonina* sp. 5 (Fig. 14).—From flower-bud galls on *E. pauciflora*, Mount Kosciusko, N.S.W., June, 1935; collected by M. J. Mackerras. The dorsal shield is a more complicated structure than any of those described already. There is a thin sheet of chitin on the 3rd thoracic segment, which thickens towards the point of junction with the 1st abdominal segment and forms a concave plate there. At the anterior edge of the 1st abdominal segment a convex chitinous hump covered with small protuberances is hinged to the chitinous concave plate described above. Any curling movement of the larva caused the convex portion to fit into the concave, but the function is unknown. The concave plate is repeated at the posterior portion of the 1st abdominal segment, and into it fits a convex portion from the anterior edge of the 2nd abdominal. Both sets are loosely connected by thin plates of chitin which have a reticulated structure. The spiracles of this larva are neither so prominent nor so complicated in their structure as any of the other larvae described. Length of full-grown larva, 4·3 mm. approx.; width of full-grown larva, 1·5 mm. approx.

11. *Fergusonina* sp. 6 (Fig. 15).—From axil-bud galls (Fig. 31) on *E. maculata*, Bateman's Bay, N.S.W., July, 1934; galls collected by Dr. Jacobs. The adult fly has not yet been reared. The larva is pearly-white with a well-defined dorsal shield. This shield is formed of four small dense plates of chitin in pairs, as in the species just described, but easily distinguished from them by the irregular area of coalesced chitinous spots surrounding the plates. Outside this area of less dense coalesced spots of chitin are isolated spots of chitin on the 2nd and 3rd abdominal segments. This structure presents a possible transitional stage between the separated plates of *F. eucalypti* Mall. and the coherent shield made up of plates all fused together in *F. nicholsoni* Tonn. The dorsal shield, measured at the widest point of the irregularly fused chitin, is about one-fourth the width of the larva at its widest point, and is about the same length as it is wide. Length of full-grown larva, 2·8 mm.; width of full-grown larva, 1·6 mm.

12. *Fergusonina* sp. 7 (Fig. 16).—From the leaf and leaf-stem galls on *E. Stuartiana*, Black Mountain, Canberra, F.C.T., March, 1935; collected by the author. The dorsal shield of this larva is composed of four loosely-joined plates which give the effect of a single plate. There are rows of strong black spines surrounding the shield on the 1st, 2nd, 3rd and 4th abdominal segments and on the 3rd thoracic segment, all the spines pointing inwards towards the shield. The shield measures approximately 0·4 mm. in width and 0·4 mm. in length. There is an extra plate on some specimens on the 3rd abdominal segment near its anterior margin. Length of full-grown larva, 2·1 to 2·3 mm. approx.; width of full-grown larva, 1·2 mm. approx.

The galls which this species inhabits are quite different from leaf galls on the same tree harbouring *F. carteri*.

13. *Fergusonina nicholsoni* Tonn. (Figs. 4 and 16A).—From flower-bud galls on *E. macrorrhyncha*, Canberra, F.C.T., November, 1930; collected by the author. The 3rd instar larva of this species has been described in some detail earlier in this paper, but it fits here into its place in the evolutionary series based on the complexity of the dorsal shield. All the plates which, in species described earlier, were separate, are fused in this larva to form a coherent shield. This shield is set on the top of a hump formed by the slightly protruding dorsal segments. The thoracic tubercles are not developed in this larva, as it lives in a cavity into which it fits tightly. Length of full-grown larva, 1·3 mm.; width of full-grown larva, 0·9 mm.

14. *Fergusonina newmani* Tonn. (Fig. 17).—From leaf-bud galls on *E. gomphocephala* DC., Perth, Western Australia, August, 1933; collected by the author. The 3rd instar larva of this species has the chitinous shield developed as in the foregoing species, but in addition, rising from the point of junction of the 1st and 2nd abdominal segments on the shield, there are two strong black hooks. These hooks appear to be used for tearing down the walls of the gall chamber for food, and their number may vary from two to three in this species of larva. The thoracic tubercles are longer than they are on the two species last described. Length of full-grown larva, 1·9 mm.; width of full-grown larva, 1·0 mm.

15. *Fergusonina brimblecombei* Tonn. (Fig. 18).—From flower-bud galls of *E. crebra*, Queensland; *E. melanophloia*, Queensland; *E. odorata*, South Australia; and *E. hemiphloia*, Victoria, June and July, 1934; collected by A. Brimblecombe, J. W. Evans and W. W. Morgan. In the 3rd instar larva of *F. brimblecombei* Tonn. the chitinous shield is similar in shape to that of the preceding species, but in addition to two hooks, which are also present in this larva, there is a strong

scoop-like projection rising from near the base of the hooks. This projection rises to about one-third of the height of the hooks. Small tubercles are present on the thoracic segments. Length of full-grown larva, 1·3 mm.; width of full-grown larva, 0·8 mm.

16. *Fergusonina lockharti* Tonn. (Fig. 19).—From globular, irregular, stem-tip galls on *E. rudis* Endl., Mundaring, Western Australia, August, 1933; collected by the author. In the 3rd instar larva of *F. lockharti* Tonn. the dorsal shield is relatively larger than that of the preceding species and the hooks which rise from the shield near the posterior margin of the 1st abdominal segment are shorter and stouter. There are three to five hooks in this larva, the usual number (as illustrated) being four. Length of full-grown larva, 2·2 mm.; width of full-grown larva, 1·2 mm.

17. *Fergusonina tillyardi* Tonn. (Figs. 20, 21).—From flower-bud galls on *E. Blakelyi*, Canberra, F.C.T., *E. camaldulensis*, Victoria and South Australia, and *E. tereticornis*, Victoria. The 3rd instar larva of *F. tillyardi* Tonn. has the most complex dorsal shield of the whole series. From the strong dorsal plate rises a rake formed of hooks, the number of these varying from five to seven. The relative size of the structure can be seen from the illustration. Figure 20 shows the larva newly moulted into the 3rd instar, so that the proportions of the mechanism relative to the body are exaggerated. Figure 21 shows a side view of a larva nearly full grown, so that the disproportion is not so great. Length of full-grown larva, 2·6 mm.; width of full-grown larva, 0·85 mm.

Function and Evolution of Dorsal Shield.

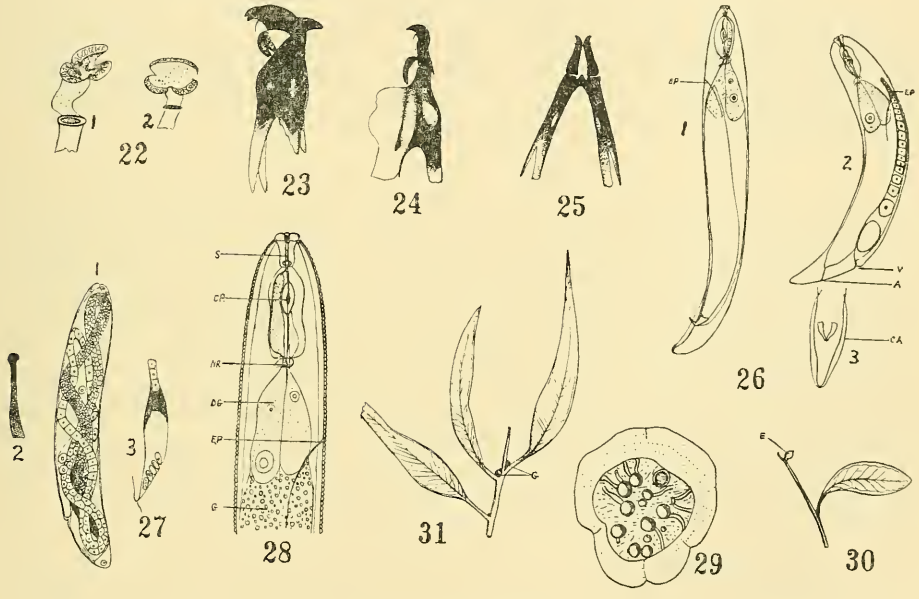
The first and second instar larvae, which feed on the contents of the soft cells lining the inner wall of the gall cavity, do not carry a dorsal shield, nor is such a shield known from any related dipterous larvae. Observation of the third instar larvae shows clearly that those larvae which possess hooks on the shield use them to tear down the walls of the galls, which begin to harden at this stage, for food. The function of this chitinous armature on the types which are not armed with hooks is unknown. The author has observed, however, that the 3rd instar larvae described above in *Fergusonina* sp. 7, from the leaf-stem galls of *E. Stuartiana*, void their faeces continually on to the dorsal shield. The nematodes are found concentrated there and apparently feeding on the faeces, but, owing to the quantity, not able to keep pace with the supply.

Faeces are practically absent in the galls made by other species of the same genus of flies, so the assumption is that the nematodes remove them as they are produced. The voiding of the faeces on the dorsum, and the feeding of the nematodes there, may be connected in some way with the presence of the chitinous patches forming the shield. When a larva bends its head backwards the axis of the body is the 1st abdominal segment and the parts just in front and behind this. This is the area which carries the dorsal shield, so that although the association between the defaecation on the dorsum, the nematodes feeding, and the presence of the chitinous shield is conjectural, it is at the same time highly suggestive.

Shields ranging from the scattered conical projections found on *F. curriei* Tonn., through the various stages of increasing complexity to the highly specialized rake carried on the dorsum of *F. tillyardi* Tonn., form a linear series which may indicate the direction of evolutionary development. In general, the types of galls formed by the more advanced larvae (as judged by the complexity of the dorsal shield) are of a more complex nature than those of the more primitive types. The relationship with the nematodes, which are found always associated with the

larvae, and which, in all cases so far studied, are transmitted by the adult female flies from one gall to the next, is also more highly adjusted in most of the species having the more advanced type of dorsal shield than in those carrying the simpler shields.

The simpler types of shield are found on larvae inhabiting leaf, stem, and stem-tip galls, while the more complicated shields are found mainly on larvae which inhabit galls on the flower buds. Purely as a matter of conjecture based on observations of these larvae, it seems that the primitive ancestor of these forms was a tunneller in the vegetative parts of the *Eucalyptus* trees. Scattered spots of chitin were replaced in some species by more and more coherent plates of chitin situated at the points of junction of the last thoracic and the first abdominal segments and of the 1st and 2nd abdominal segments. Having developed into a coherent shield, the next step forward to the development of the rake on the shield is more easy to interpret on the basis of function.



Text-figures 22-31.

22.—Anterior spiracle of larvae of *Fergusonina* spp. (1, *Fergusonina* No. 4; 2, *F. tillyardi* Tonn.), × 100.

23.—Mouth parts of *F. tillyardi* Tonn., × 60.

24, 25.—Mouth parts of *F. eucalypti* Mall. (24, × 60; 25, dorsal view, × 60).

26.—*Anguillulina* (*Fergusobia*) *tumifaciens* (1, male; 2, free-living female; 3, male, ventral view of anal end. × 100). V, vulva; A, anus; E.P., excretory pore; C.A., caudal alae.

27.—Parasitic female (1, side view; 2, tip of ovary; 3, oviduct expanded forming a receptaculum seminalis. Mature eggs ready to be laid lying in uterus. × 40).

28.—Anterior end of free-living female of *A. tumifaciens*. × 270. S, stylet; O.P., oesophageal pump; N.R., nerve ring; E.P., excretory pore; D.G., dorsal gland; G, gut.

29.—Cross-section of galled flower-bud of *E. macrorrhyncha*. Semi-diagrammatic, showing stalked gall-lets inside the galled flower bud. × 2.

30.—Gall on *E. polyanthemos*. E, point at which fly will emerge. × 0·2.

31.—Axil bud galls on *E. maculata*. G, galls. × 0·2.

The great variety of *Eucalyptus* species provided opportunity for variation in the flies. Adaptation to galls which harden and dry out fairly rapidly at maturity would be successfully attained by species which developed a mechanism to tear down the hardening tissues for food. Forms carrying hooks would survive more readily than others not so well equipped.

It is necessary here to point out that the degree of adaptation of the larva to a special environment need not be, and indeed is not, reflected in the adult fly which has to meet a totally different set of conditions. Tonnoir, in describing the adult flies, could not find any characters which would suggest that the flies from the more primitive types of larvae were more primitive in structure than those from the more highly adapted larvae. The larval environment induces reaction in structure which have no apparent counterpart in the structure of the adult fly.

II. *The Associated Nematode.*

The nematodes described below were taken from leaf galls of *E. Stuartiana* in which they were found associated with *F. carteri* Tonn. They were chosen because large numbers of them were available at a time when it was convenient to study them in the laboratory. The parasitic female nematodes were derived from the body cavities of females of *F. carteri* Tonn.

Anguillulina (Fergusobia) tumifaciens, n. subgen. et sp.

The egg (Pl. vii, fig. 4).—There is no apparent difference between the eggs laid by the free-living females in the gall and the parasitic females inside the *Fergusonina* flies. The eggs of both types of females are already segmenting when laid and, prior to hatching, the larva can readily be seen moving round inside the shell. The outer skin of the egg is tough and translucent, without sculptural markings, and its shape is nearly cylindrical with both ends rounded. Length of egg, 0·05 to 0·055 mm.; width of egg, 0·018 to 0·025 mm.

The larva (Pl. vii, fig. 4).—The newly-hatched larva measures 0·14 to 0·19 mm. long and 0·009 to 0·011 wide. It has a well-developed stylet carrying three basal swellings and measuring 0·006 mm. in length. The number of larval instars has not been determined accurately, but at least four instars are discernible.

The adult.—The free-living form (actually a plant-parasite in the leaf galls of *E. Stuartiana*) may be treated as the normal form and will be described first.

(*a*). Male (Fig. 26, 1).

Principal measurements: Length, 0·415 mm.; width, 0·049 to 0·05 mm.; anterior end to end of oesophageal region, 0·124 mm.; anterior end to excretory pore, 0·089 mm.; anus to tip of tail, 0·04 mm.; length of buccal stylet, 0·012 mm.; length of spicules, 0·021 mm.; proportions: length to breadth, 8–9:1; length to length of oesophagus, 3–4:1; length to length of tail, 10–12:1.

The cuticle is finely striated transversely. The head is separated from the body by a very slight constriction, has rounded sides, and shows no sign of separate lips or papillae. The body tapers slightly towards the head from the posterior end of the oesophageal region, but tapers much more abruptly towards the tail. The oesophageal region is well defined. The dorsal pharyngeal cell is very prominent, with a large nucleus, and opens by a short duct just behind the stylet. No gubernaculum has been seen in any specimen examined. The paired spicules are in contact distally, but are separated at their proximal extremities. They show a distinct elbow bend when viewed laterally and taper to a rounded point. The gonad is unpaired and in mature specimens it can be seen lying with

its origin in the oesophageal region, thence running backwards without reflexing to open in the cloaca. The tail tapers to a rounded point. The cuticle is expanded laterally to form two alae arising well in front of the cloaca and extending round the tail tip (Fig. 26). The margins of the alae are slightly crenate.

Spermatozoa: Under high magnification the globular spermatozoa were observed to possess a number of processes which looked like amoeboid strands with their distal portions slightly clubbed. When stains were applied the globular portion took up the stain, but the strands did not do so, or did so to a less extent.

(*b*). Free-living female (Fig. 26, 2).

Principal measurements: Length of body, 0·415 mm.; width of body, 0·056 mm.; length, anterior end to end of oesophageal region, 0·123 mm.; length, anterior end to excretory pore, 0·09 mm.; anus to tip of tail, 0·039 mm.; vulva to tip of tail, 0·08 mm.; buccal stylet, 0·019 mm.; proportions: length to breadth, 7–8:1; length to length of oesophagus, 3–4:1; length to length of tail, 10–11:1.

The female is more bluntly rounded anteriorly than the male, but tapers more rapidly posteriorly. The head and oesophagus are similar to those of the male. The single ovary arises near the nerve ring and opens into an oviduct in which there is no post-vulval pouch. One to three fully developed eggs may lie in the oviduct. The lips of the vulva are prominent in mature females but not in immature specimens.

(*c*). Parasitic female (Fig. 27).

Principal measurements: Length of mature female, 0·69 to 0·87 mm.; width of mature female, 0·12 to 0·14 mm.; length of ovary removed from body and stretched out straight, 3·0 mm.; vulva to posterior tip of body, 0·15 to 0·22 mm.

When the fertilized female enters the haemocoel of the fly larva, she carries the stylet and oesophagus of the free-living form. Growth is rapid, however, the female soon loses the stylet, and the gut shrinks as the rapidly expanding, much coiled, ovary grows to fill almost entirely the whole space inside the skin. Just under the cuticle in this stage a layer of polygonal pavement cells can be seen, which, according to Goodey (1930), allow a great increase in size without further moulting.

Some of the parasitic females could flex their bodies until head and tail nearly met, while in others the power of movement had been lost entirely. Those from the body of *F. nicholsoni* retained the power of movement only up to the stage at which egg-laying commenced; after that, movements of the body as a whole ceased.

Development of the Parasitic Nematode in the Flies.

The parasitic female nematodes live in the haemocoel of the larval, pupal, and adult female flies. On entering the female fly larvae the nematode still possesses all the free-living characters, including the stylet (Pl. vii, fig. 7). About the time at which the fly larva pupates the nematodes grow rapidly, lose their stylet, and develop a great reserve of food, before their ovaries become apparent (Pl. vii, fig. 10). At the stage when the fly larva has pupated, the nematode female can be seen lying in the finely disintegrated fat-body of the insect with multitudes of the small particles of the fat-body so firmly adherent to the outside of the skin that it requires some force to remove them. At this stage the ovary of the nematode develops and becomes differentiated at a very rapid rate, while the ovary of the fly is only beginning to develop. It looks as if the same factors which lead to the growth of the fly ovary have caused a parallel rapid develop-

ment in the nematode ovary. The nematode ovary develops much more rapidly than that of the fly, however, so that, before the eggs of the fly can be seen taking shape in the ovarian tubules, the nematode has started to lay eggs (Pl. vii, fig. 12). By the time eggs can be seen in process of formation in the ovary of the nematode, the granules of fat-body of the host insect are no longer adherent to her skin, and the fat-body of the fly has become reorganized into large compound globules.

The adult *Fergusonina* flies do not feed to any great extent, though they have been seen sucking up moisture, so it is evident that the fat-body of the fly has to supply all the nutriment for the growth of its own ovary (it is mature at emergence) and that of its contained nematodes. It is likely that the parasitic nematodes affect the egg-producing capacity of the fly in proportion to the number and size of individuals present. It happens, therefore, that, in the example dealt with in detail (*Fergusonina nicholsoni*), where only two nematodes are found normally in each female fly, an equilibrium has been reached at which the egg-laying power of the fly may be depressed, but is not impaired seriously. In other flies of the same genus, usually of larger size, the nematodes are, in some instances, bigger and more numerous. As many as seven large nematodes have been dissected out from a female fly of a leaf-gall species in which the ovaries were found to be complete, but, owing to the scarcity of fat-body, not capable of producing many eggs. The larval nematodes in the fly probably feed to some extent in their turn, but not enough to interfere materially with the development of the eggs of the fly.

The sequence of events just described forms a contrast with the state of affairs found by Goodey in his study of the Frit fly–nematode association. He found that the presence and development of the parasitic nematodes in male and female flies inhibited the development of the gonads of the hosts. When the host was able to develop its gonads to some extent before the influence of the nematodes could be felt, then the further development of the nematodes was checked—presumably by some inhibiting factor produced by the developing gonad. Just such a difference in the physiological reactions between Frit fly and *Eucalyptus*-gall fly, to a nematode parasite, might go far to explain why in the former case a destructive parasitic association only is reached, whereas in the latter, a symbiosis has been attained.

Nematodes Associated with Other Species of Fergusonina.

It is probable that further work will show that the nematodes associated with the different flies have differences in structure which entitle them to be considered as different species. There is, for instance, a considerable variation in the point of origin of the lateral alae between males derived from different types of galls. On males from the leaf galls of *E. macrorrhyncha*, the alae extend from a point opposite the nerve ring to the tail tip, whereas in the species just described, they are much less extensive. The position and size of the oesophageal glands also vary between nematodes from different galls and the parasitic females vary considerably in size in the different species of flies. The largest parasitic nematode female discovered, taken from females of *F. curriei* Tonn., measured 2.16 mm. long by 0·166 mm. broad.

Taxonomics.

The well-developed buccal stylet (Fig. 28) and the plant-parasitic habit, clearly indicate that the nematode which is being described has origin and affinities with

the plant-parasitic nematodes. A much enlarged parasitic female phase occurs in a number of nematode genera found in insects, so a consideration of each of those described previously is necessary for comparison with *Anguillulina* (*Fergusobia*).

(*a*) *Tylenchenema* Goodey has no buccal stylet in the male, so this leading feature may be regarded as clearly separating the new nematode from that genus.

(*b*) *Sphaerularia bombi* Dufour and (*c*) *Atractonema gibbosum* Leuckart are totally different in the form of the parasitic female.

(*d*) *Howardula benigna* Cobb.—The life history of *Howardula* resembles that of the new nematode in some particulars (Cobb, 1921), but the "onchium" which Cobb describes as non-bulbous is a good character for definite differentiation.

(*e*) *Allantonema* Leuckart and (*f*) *Bradynema* von Strasson have been considered as synonyms by Bayliss and Daubney. The paired, opposed, reflexed ovary of the free-living females of these genera serves to distinguish them from the new nematode.

The nematodes from *Eucalyptus* galls can, therefore, readily be distinguished from other known nematodes parasitic in insects, but they have strong affinities with the plant-parasitic group, so some consideration must be given to the genera in that group which they approach most closely.

(*a*)* *Heterodera* Schmidt.—*Anguillulina* (*Fergusobia*) can be distinguished from *Heterodera*, the former having a single ovary, contrasted with the paired ovary of *Heterodera*. In addition, the dorsal gland of *Heterodera* opens at some distance from the base of the stylet, while in the other nematode the opening is close to the base of the stylet.

(*b*) *Aphelenchus* Bastian.—There are no knobs on the base of the stylet in this genus, a feature which serves to distinguish it from *Anguillulina* (*Fergusobia*).

(*c*) *Aphelenchoides* Fischer.—In this genus the opening of the oesophageal gland is at some distance from the base of the stylet and the spicules are thorn-shaped.

(*d*) *Anguillulina* Gervais and v. Benedin.—There are many characters, such as the knobbed stylet, the position of the opening of the dorsal oesophageal gland, the caudal alae of the male, and other characters common to both, which show affinities between the new nematode and *Anguillulina*. There are also characters such as the absence of a gubernaculum in the nematode under discussion, and the presence of an inflated degenerate female as a phase of the life-history cycle, which suggest differences almost of generic rank. In order to indicate the supposed origin and affinities of the nematode the generic name *Anguillulina* is applied, but to show the relationship with flies of the genus *Fergusonina* the name *Fergusobia* is suggested as a subgeneric title.

Diagnosis: Anguillulina (Fergusobia), n. subgen.

Plant-parasitic forms.—Small slender worms, sub-genotype described in the foregoing, about 0·4 mm. long, living in leaf galls of *E. Stuartiana* in association with larvae of flies of the genus *Fergusonina*.

♂. With well-developed stylet, the anterior part cylindrical, the base with three distinct swellings. Tail tapering behind anus and bearing caudal alae. Spicules paired and shaped like those of *Anguillulina*. Gubernaculum absent. Testis single, anterior.

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* Generic characters of these genera are from Goodey, 1933.

♀. Stylet as in male. Ovary single, anterior, without post-uterine sac. Oviparous, 1 to 3 mature eggs visible in oviduct at one time, eggs segmenting when laid, sometimes ready to hatch. Free-living female in galls parthenogenetic in first generation.

Insect-parasitic forms: Sausage-shaped, much enlarged females with greatly developed ovary much coiled and filling nearly whole body space. Stylet absent in mature female. Spermatozoa visible in widened portion of uterus which acts as a receptaculum seminis. Oviparous, eggs segmenting when laid, and any number up to ten lying mature in oviduct ready to be laid.

The sub-genotype is parasitic in the body cavity of *F. carteri* Tonn. The measurements have already been given above in descriptions of stages.

Types of Association Between Nematodes and Insects.

Various types of association between nematodes and insects have been described and all instances recorded before 1927 have been collected into one publication by Van Zwaluwenburg (1928). He recognizes five types of association as follows: (1) Primary parasitism; (2) secondary parasitism; (3) internal mechanical association; (4) external mechanical association; (5) commensalism.

The nematode association with flies which we are considering does not fall into any of the foregoing categories, but it resembles, to some extent, the type of association described by Goodey (1930) between *Tylenchenema oscinellae* Good. and the Frit fly *Oscinella frit* L.

The association in the *Eucalyptus* galls is not purely parasitic, however, as it is in the example cited above, but may be regarded as a symbiosis which may have developed from a pre-existing parasitism.

Origin of Fly-Nematode Association.

The data available warrant some speculation on the probable origin of the association between the flies and nematodes in *Eucalyptus* galls.

The close affinities with modern plant-parasitic forms make it almost certain that the ancestors of the nematodes in the galls were plant parasites, and probably gall-formers closely related to *Anguillulina*.

The ancestors of the associated flies (*Fergusonina* spp.) were probably leaf-miners (leaf-mining forms are common in the family Agromyzidae), living first in the leaf and, later, in the leaf and stem-tip tissues, as these are less specialized and more available throughout the year than the flower buds. At first the association between the fly and nematode would be of an accidental type resembling the association of the nematode and Frit fly already mentioned. In such an accidental association, eventually some nematodes would penetrate the body of the fly larva, and in the highly nutritious and easily assimilable food medium, develop an enlarged and degenerate type of female, capable of producing a great number of eggs; there are many examples in the animal kingdom of this reaction to the parasitic mode of life. This very large increase in egg-laying power, together with the automatic transmission of the progeny of such a female from one generation of buds to the next, could well explain the survival of the nematodes which had developed this stage in their life history. A necessary condition for the survival of such an association, if it were to affect all of the flies, would be that the nematodes should not render their hosts totally infertile.

The foregoing sequence of events would explain the survival of those nematodes which developed this association with flies, but it does not explain why the flies containing nematodes could survive, rather than the flies acting

alone. In nature at the present time the association is complete, *all* fly-galls discovered up to the present containing nematodes.

It is necessary, therefore, to examine the effect of the association on the fly. In the forms studied in the present paper it was discovered that the nematodes were actively feeding and causing proliferation of the plant tissues around the egg of the fly before it hatched; and that when the fly larva did hatch, it fed on those abnormal tissue growths. This suggests the value of the nematode to the insect. From what was probably a leaf-mining form, the fly developed into a gall-living form, finding, on hatching, a suitable and easily available pabulum already provided for its first needs through the activities of the larval nematodes. A slightly higher survival rate for the newly-hatched larvae associated with the nematodes than for the larvae in plant tissues not associated with nematodes would lead ultimately to the displacement of the latter "free" types by the former "associated" types. Once the association had become established in the vegetative parts of the trees, some flies could readily attack the flower buds and establish themselves there to give rise to new species in time. Refinements in the adjustments of the nematode to the fly and of both these to the galled tissues would naturally follow. Some of the trends of these refinements are indicated by the following notes. In *Oscinella frit*, Goodey found that the nematode rendered its host infertile and that a percentage ranging from 1% to 14·7% of the flies were infected. Nematodes were found to enter and to render infertile both males and females. In all the species of *Fergusonina* the nematodes are found in the galls associated with both male and female larvae, but they are never found in the parasitic form in the male adult flies, and are always found in the female adult flies.

In the "lower" types of association, as illustrated by leaf-gall forms of *Fergusonina*, the number of female nematodes in an adult female fly may vary from three to seven, whereas in the flower-bud form already described in detail the number of parasitic nematodes is almost consistently restricted to two. There is therefore considerable evidence to support the view that the association of flies and nematodes in the galls of *Eucalyptus* trees was at first an accidental parasitism which has now developed into a symbiosis, and that the association existing at the present day in galls on the vegetative parts of the trees represents a slightly more primitive stage in the development of the symbiosis than that in the galls on the flower buds.

III. *The Galls.*

Gall Formation in E. macrorrhyncha.

The flower buds generally appear in January and February on trees of this species, although there may be some variation both in the time and the amount of flower buds which the trees produce, especially under erratic rainfall conditions. The buds appear about the same time as the adult flies of *Fergusonina nicholsoni* are leaving the mature galls. The punctures made by the ovipositors of the female flies can be seen for some days after they have been made, but they do not seem to cause any special distortion of the tissues by the mechanical rupturing of the cells in their path. Eventually they heal and show only a slight scar on the operculum where they were made.

Immediately they enter the bud cavity, the larval nematodes start to feed on the ring of anther primordial cells which form a circle round the inner wall of the bud cavity. The anther primordia proliferate rapidly, forming shapeless masses of large, thin-walled, parenchymatous cells, full of mucilaginous cell-sap.

The outer wall of the bud grows in proportion to the inner proliferation, so that about a month after the nematodes and the eggs of the fly have been deposited in the buds, the galled buds can be distinguished from the ungalled buds. By the time the eggs of the fly hatch the masses of galled tissues are already present in the bud.

Evidence that the nematodes start the galling before the eggs of the fly hatch was found in the following observations. In some galled buds which were opened it was found that the fly eggs were infertile (the embryo is easily seen in fertile eggs) but the galling had already been started by the nematodes. In all such cases the galls aborted at an early stage, while the nematodes failed to reproduce and died after some months. No fly larvae have yet been found unaccompanied by nematodes, so that the fate of a fly larva, if unaccompanied by nematodes, could not be observed. Attempts to inject living nematode larvae artificially into flower buds failed.

The photo-micrograph (Pl. vi, fig. 8) of a longitudinal section of a galled flower-bud of *E. macrorrhyncha* shows the state of affairs before the egg of the fly hatches. The masses of tissue proliferating under the stimulus of the nematode irritation can be distinguished clearly.

On hatching, the fly larvae ensconce themselves between two firmly apposed masses of tissues, and there cut out small crypts. The larval nematodes migrate to these crypts to join the fly larvae, and thenceforward the fly larvae and their accompanying nematodes are to be found only in these crypts, and always together. In a single galled flower-bud there may be twenty or more of these gall-lets, each with its single fly larva and associated nematodes (Fig. 29). The crypt becomes a separate unit by the fusing of the vegetable tissues around the larva to make a spheroidal gall-let and the gall-let is attached to the wall of the bud by a strong stalk through which nourishment passes to the developing cells. A section through one of these gall-lets during the second instar period of the larva (Pl. vi, fig. 12) discloses the following formation: A distinct wall of flat cells forms the outer covering, and inside these are many layers of thin parenchymatous cells, well filled with protoplasm and mucilaginous cell-sap. On the inner surface, which is in contact with the nematodes and the fly larva, the cells are small and full of protoplasm. From these cells a mucilaginous fluid is exuded into the cavity of the gall.

This state of affairs persists through the autumn, winter, and early spring, but with the onset of summer conditions, the galls begin to mature. The outer layer of flat cells on the gall-lets becomes somewhat lignified and when the larva enters its third and final instar, it tears down the inner layers for food. At this stage the free-living nematodes are destroyed by the drying up of the gall tissues, but fertilized females have already entered the female fly-larvae.

Plate vi, figures 6–9, are photomicrographs of the different stages in the growth of the galls, while Plate vi, figure 10, shows a cross-section of a normal, ungalled flower-bud.

The photomicrograph (Pl. vi, fig. 6) shows a section of a flower bud of *E. macrorrhyncha* measuring about 1 mm. across at its widest part. Eggs had been laid in this bud by the fly, three days prior to fixation. At A can be seen the outer layer of the operculum or bud cap which, morphologically, represents the sepals. At B the inner layer of the operculum, which is considered to represent morphologically the petals of a complete flower, can be distinguished. This cap is shed at flowering, the remaining flower consisting of a multiple ring of stamens

with long filaments supporting the anthers, arising from near the lip of a cup above the ovary. At C in the section can be seen the ring of primordial tissues which give rise to these stamens. Lying in the cavity between the operculum and the base of the bud are a number of larval nematodes, N, which have already started to feed on the rapidly developing anther primordia.

Plate vi, figure 7, illustrates a more advanced stage in the development of the gall. Large irregular masses of cells can be seen proliferating into the bud cavity, where normally the stamens should be developing regularly.

Plate vi, figure 8, shows a still later stage in development of the gall. About this stage the eggs of the flies hatch and the larvae proceed to ensconce themselves between two masses of cells which are in contact. The cell masses anastomose round the crypts containing the larvae of the flies and the nematodes which have joined them. The cells immediately surrounding the larvae differ somewhat from the rest; the cell contents have a mucilaginous character, while more distant cells are more watery; likewise, the layers just round the cavity are full of protoplasm and are meristematic in character. Plate vi, figure 9, represents a still later stage in the development of the gall. At this stage the bud can be recognized from external examination to be definitely galled when compared with the normal bud. Large masses of galled tissues fill the bud cavity. Plate vi, figure 10, is a photograph of a longitudinal section of a normal bud of exactly the same age as the bud shown in Figure 9. The normal stamens can be seen lying in the bud cavity, the filaments in the upper part, and the anthers under them, while the ovules are seen in the chamber of the ovary below.

All the sections described so far have been longitudinal; the following are cross-sections: Plate vi, figure 11, illustrates a galled bud about six months old. This bud was chosen because it contained but a single gall-let and some of the stamens developed normally. This gall-let is cut through in the section at a point where the larva of the fly was not included. Nematodes can be seen lying in the cavity. In Plate vi, figure 12, the most common condition of a galled bud six months old is shown in cross-section. On this section thirteen gall-lets have been cut through at different points, eleven of them being *Fergusonina*, the other two larval wasps, shown at 1 and 4. Plate vi, figure 13, shows a single gall-let of *Fergusonina* and a single wasp chamber, for purposes of contrast.

At A can be seen the dense protoplasm-filled cells surrounding the nematodes and the fly larva. An ooze of glutinous fluid covering the surface of the cells can also be distinguished in the photograph. At B the cells are fairly dense, somewhat hexagonal, soft, and filled with clear, slightly glutinous cell sap. At C there is a layer of thick-walled cells which form the outer wall of the dipterous gall-let. They contain some brown coloration and are not sclerotized at this stage. At D is seen the larva of the fly, shrunken from the wall of the cavity by the fixing and dehydration processes. The nematodes can be seen at N surrounding the larva.

When the larva has finished feeding, only the outer lignified shell of the gall-let remains. Galled buds vary in size with the number of gall-lets contained, the normal mature flower bud measuring about 3 mm. in diameter, while a galled bud may be 14 mm. The average size of 200 galled buds of *E. macrorrhyncha* containing fly larvae and no hymenopterous insects was 5·7 mm. When hymenoptera are present as well, the galls are much larger, the average diameter for 300 galled buds containing both diptera and hymenoptera being 9·3 mm.

Comparison of wasp galls and fly galls.

Many galls were found on flower buds of *Eucalyptus* trees which had been formed by small hymenoptera alone, while in other instances both hymenoptera and diptera occupied the same galled buds. The short discussion which follows deals with galled buds on *E. macrorrhyncha*, which contained at the same time larvae of *Fergusonina* spp. and small hymenopterous gall-forming species.

It is of some interest to compare the type of gall tissue produced by the gall wasps with that produced by the fly larvae. This comparison will be restricted to tissues produced in the same galled bud by the two organisms, as the wasps frequently oviposit in buds already beginning to show signs of the galling caused by the flies. The cross-section (Pl. vi, fig. 13) shows, in juxtaposition, tissues galled by the wasp and those galled by the fly. The wasp larva produces a gall with a thick outer wall of heavily lignified cells and an inner mass of large thin-walled cells of spongy parenchyma, full of a watery cell-sap, and the cavity in which the larva lives is very much larger than the larva itself.

Whereas it is considered that the beginning, and a good part of the later growth, of the *Fergusonina* gall is caused by the nematodes, the wasp gall starts to form before the egg of the wasp hatches. Apparently there is some chemical stimulus to gall production, either in the egg itself or in a fluid injected by the parent wasp during oviposition. The gall reaches its full size before the larva has passed its first instar so that, after active feeding commences, there is little increase in the size of the gall, though there is probably a change in the composition of the cell contents.

Many gall-forming wasps appear to inject a fluid with their eggs, which causes the formation of the gall, while in other instances the egg itself, either by its mechanical properties or some chemical on its surface, gives origin to the galling. In the flower buds of *E. macrorrhyncha* galling started where nematodes were actively feeding and not particularly round the eggs of the flies. Moreover, the character of the gall changed after the fly larva had hatched and commenced to feed, so that apparently the first proliferation of cells was caused by the nematodes working alone, and the later development by the nematodes and fly larva working in conjunction. The ultimate cause of gall making, be it chemical or mechanical, has not been studied and no theory to explain it is advanced here. For theories on this subject, see Goodey (1935).

Gall Formation in Other Eucalyptus Trees.

Having treated the galls on *E. macrorrhyncha* in some detail, a little must be said about gall formation in other *Eucalyptus* trees and on situations other than the flower buds. The following table sets out the species of the genus *Fergusonina*, the larvae of which have been described in the present paper, and the species of adults which have been described by Tonnoir (1937), together with the species of trees and the situations on which they have been found.

TABLE 1.—(*Genus*) *Fergusonina*.

Species.	*Host.*	*Galls.*	*Adult.*	*Larva.*
microcera Mall. (*Genotype*)	—	—	+	—
atricornis Mall.	—	—	+	—
flavicornis Mall.	—	—	+	—
scutellata Mall.	—	—	+	—
bisecta Mall.	*E. maculata*	? flower-bud	+	—
gurneyi Mall.	*E. maculata*	? flower-bud	+	—
eucalypti Mall.	*E. maculata*	flower-bud	+	+

TABLE I.—*Continued.*

Species.	*Host.*	*Galls.*	*Adult.*	*Larva.*
carteri Tonn.	*E. amygdalina*	?	+	+
	E. Stuartiana	leaf		
evansi Tonn.	*Eucalyptus* sp.	leaf	+	+
davidsoni Tonn.	*E.* sp.	? leaf	+	—
brimblecombei Tonn.	*E. melanophloia*	flower-buds	+	+
	E. hemiphloia	,,		
	E. crebra	,,		
	E. odorata	,,		
morgani Tonn.	*E. hemiphloia*	flower-bud	+	—
pescotti Tonn.	*E. amygdalina*	leaf	+	—
newmani Tonn.	*E. gomphocephala*	leaf-bud	+	+
lockharti Tonn.	*E. rudis*	stem-tip	+	+
frenchi Tonn.	*E. amygdalina*	leaf	+	—
nicholsoni Tonn.	*E. macrorrhyncha*	flower-bud	+	+
curriei Tonn.	*E. macrorrhyncha*	leaf	+	+
tillyardi Tonn.	*E. Blakelyi*	flower-bud	+	+
	E. camaldulensis	,,		
	E. tereticornis	,,		
greavesi Curr.	*E. polyanthemos*	stem-tip	—	+
sp. 1	*E. pauciflora*	stem-tip	—	+
sp. 2	*E. macrorrhyncha*	stem-tip	—	+
sp. 3	*E. sideroxylon*	leaf	—	+
	E. maculosa	,,		
	E. melliodora	,,		
	E. macrorrhyncha	,,		
sp. 4	*E. maculosa*	leaf	—	+
sp. 5	*E. pauciflora*	flower-bud	—	+
sp. 6	*E. maculata*	axil-bud	—	+
sp. 7	*E. Stuartiana*	leaf and leaf-stem	—	+

The flower buds of *E. Blakelyi*, *E. camaldulensis*, and *E. tereticornis* are all galled by the same species of fly, *F. tillyardi* Tonn., and the galling proceeds as follows:

The nematodes start the galling and the fly larvae hatch out afterwards and commence to feed, then the nematodes join them in their small crypts and the galled buds increase in size. Whereas in *E. macrorrhyncha*, the crypts are each separated into gall-lets by the formation of a layer of flat cells making a limiting membrane, no such membrane is formed in the flower buds of the other trees mentioned, but the whole of the tissues on the galled buds fuse together into a mass which is bounded only by the outer walls of the flower buds. Inside this more or less homogeneous matrix of soft parenchymatous tissues, and scattered through the whole, are to be found the crypts in which larvae and nematodes live. In cross-section it is seen that some layers of cells surrounding each of the larval crypts are filled with more dense protoplasm and cell-sap than the tissues of the matrix.

During the first and second larval instars in autumn, winter, and spring, the galled tissues are soft and glutinous. With the advent of summer and the larval change to the third instar, the tissues begin to harden and to dry up. When the larvae are full fed, the tissues forming the matrix between crypts dry up and the whole gall disintegrates rapidly, so that there are numerous avenues of escape for the adult flies. The photographs (Pl. vi, figs. 3 and 4) show the galled, contrasted with the ungalled, buds of *E. Blakelyi*. Galled buds of the three trees mentioned may reach a diameter of 18 mm., while the ungalled buds average about 3 mm. in diameter. The galled buds of *E. maculata* are similar in development and structure to those of *E. Blakelyi*.

Next to *F. tillyardi*, which is found on *E. camaldulensis*, *E. tereticornis* and *E. Blakelyi*, the most common fly found so far in flower buds is *F. brimblecombei* Tonn., which is found in *E. hemiphloia*, and *E. odorata*, *E. crebra* and *E. melanophloia*. The galls formed by this fly are somewhat different in structure from those formed on *E. camaldulensis*. Instead of forming a homogeneous mass inside the galled bud as in *E. camaldulensis*, the stamens in the species of *Eucalyptus* trees named above develop to some extent. The tissues which hypertrophy in this instance belong to the walls of the calyx-cup and the ovary. At maturity, the operculum, which is not fused to the rest of the gall, opens and the flies escape into the space occupied by the stamens by the break-down of the galled tissues, and thence, via the opercular opening, to the exterior. Galled buds of *E. hemiphloia* are about 8 mm. in diameter, while *E. maculata* and *E. odorata* produce galls up to 20 mm. in largest diameter.

The galls formed on leaf and stem-tip tissues are generally irregular and warty in appearance. They are simple in construction, each larva occupying an individual chamber in which it lives with the nematodes. Examples of this type are found on the leaf tips of *E. gomphocephala* and *E. maculosa*.

Another type of gall is formed on the shoot tip of *E. macrorrhyncha* and on *E. rudis*. This consists of a large mass of spongy tissue containing a number of larvae and surrounded by a clearly-defined, pigmented, tough outer skin (Pl. vi, fig. 5). The larvae and accompanying nematodes live near the centre of the galled mass until the former are near the point of pupation, when each larva tunnels an individual track to just under the skin, where it pupates. On the leaves of *E. Stuartiana* and other *Eucalyptus* trees, the galls are formed between two developing leaves which, on fusing together, form the upper and lower ends of a series of gall cavities (Pl. vi, fig. 5).

Regular Galls.—All the galls mentioned so far are shapeless and irregular, but two examples of regular, though not complex, galls have been observed. One of these is a stem-tip gall formed on *E. polyanthemos* (Fig. 30). The gall illustrated shows clearly, when mature, the light patch on the side which is to be the emergence hole of the adult fly. The other example is provided by the axil buds of *E. maculata* (Fig. 31). These are individual spherical galls with emergence holes prepared by the full-fed larvae before pupation.

There are no rich architectural designs produced by *Fergusonina* spp. to compare with those made by the many gall-making coccids of the genus *Apiomorpha*, so well known on *Eucalyptus* trees in Australia.

Fluctuations in the Numbers of Galls from Season to Season.

During certain years galling of the flower buds of some eucalypts was extremely heavy, particularly on *E. camaldulensis*, *E. hemiphloia*, *E. tereticornis*, *E. Blakelyi*, and *E. macrorrhyncha*. In other years considerable areas had to be searched before galls could be discovered, even when flower buds were abundant. These violent fluctuations in numbers were much more evident in the flower-bud galls than on the stem and leaf-tip galls, and the latter were never seen in such overwhelming numbers as the flower-bud galls. In examining the causes of this phenomenon it would appear that there is a more constant high level of parasitic control on the less specialized stem-tip, and leaf-tip galls, than on the relatively more specialized flower-bud galls. The incidence of parasites will be considered separately, but it is necessary to mention it here because there is no doubt that parasites exercise considerable influence in deciding the amount of galling under certain conditions.

As an actual observation of the fluctuation of the numbers of galls the sequence of events on a single tree of *E. macrorrhyncha* may be quoted:

During 1930, although buds were present in fair numbers, there were no galls formed on the tree.

In 1931 there were fewer flower buds formed but only a small number of these were galled.

In 1932 there was a heavy crop of flower buds and the galling was so complete that scarcely a bud reached the normal flowering stage. Galls were mostly of mixed origin, flies and wasps both being present.

In 1933 the galls containing wasps still hung on the tree from the previous year, but only a very small number of new buds were formed. These were galled by the flies which were then emerging in large numbers, but hardly any of the galls reached maturity.

In 1934 there were many flower buds on the tree, but none was galled.

Early in 1935 new buds formed and none of them was galled, but the tree died. The extremely heavy galling in 1932, especially the excessive number of wasp galls, which seemed to take more out of the tree because of their longer (2 year) duration, appears to have weakened the tree, which had also been attacked by a fungus, thus contributing to its death.

The setting of flower buds on *Eucalyptus* trees is not very regular, the amount of bud setting being dependent on the locality, the amount and distribution of rainfall during the previous and in the current year, and the other climatic conditions during the period. Buds may be formed every year on *E. macrorrhyncha*, or, in unfavourable seasons, scarcely any buds may form on the trees at all. Flowering takes place in the Federal Territory generally during February or March, and the young buds which will flower during the year following usually appear earlier in the year. One may frequently see, in March, a single tree carrying flowers fully out, flower buds which had set the previous January, and flower buds six months old.

The conditions necessary for heavy galling of flower buds in any one year are: (1) The trees must bear a heavy crop of flower buds; (2) large numbers of gall flies must be present near the trees (the gall flies are not strong fliers); (3) the flies must emerge from last year's galls just at the same time as the young flower-buds are appearing on the same, or on other trees.

Taking these conditions in order, we see that owing to the erratic flowering of the *Eucalyptus* trees, a year or two may pass before a heavy crop of buds appears, so that the possible total number of flies may be regulated by the number of flower buds available. In particularly bad years, buds may be so scarce that the fly population is reduced to a very low level.

The second condition (No. 2 above) has a twofold demand. First, large numbers of flies must be present in galls from the previous year, which means that the previous year must have produced a fair number of galls, and second, the flies must be within range of the new buds. It is a matter of common observation that certain localities have a somewhat different flowering rhythm from others, so that these districts may, under certain adverse circumstances, become so unfavourable for the flies that they cease to exist there, and the areas have to be slowly re-colonized from elsewhere. Speaking very broadly, it is true that whole climatic regions in Australia have regular fluctuations in the numbers of flower buds present, but, within these, smaller localities do exist which vary away from this regular sequence. As an example of the possible non-fulfilment of the condition named in (2), it was observed that a group of trees of the species

E. Blakelyi bearing many buds was untouched by galling in 1932, while only a mile away galling was heavy on another group of trees of the same species. The following year the group of trees which was carrying a heavy crop of galls produced vast numbers of flies, but no tree of that group produced any flower buds, and trees only a mile distant, which bore buds, were not galled.

The third condition is a most important one which is frequently not fulfilled. In *E. macrorrhyncha* the buds galled by the fly open to permit the escape of the flies before the normal flowering time of the trees, and just before young buds appear. The short life of the adult fly makes it imperative that the interval between emergence and the appearance of the buds should be not more than a month, so that if the tree from which the flies emerge does not bear young buds that year, then neighbouring trees must bear buds about the same time to allow survival. Normally, trees of the same species flower, and bud, about the same time, but, for reasons not as yet explained, the flowering time may be spread over a long or a short period. The ideal conditions for heavy galling in any year occur when trees bud simultaneously, and when budding corresponds to the time of emergence of the flies.

A year of heavy budding may give a heavy galling, but very seldom do successive seasons produce heavy crops of buds in most districts, so a maximum galling year produces flies which usually find the following year a restricted number of buds in which to lay, and so may be reduced in numbers that year to a minimum. This minimum is in no danger of complete annihilation as there are always some trees, or at least some branches on certain trees, which produce buds out of season, or during a season when other trees fail to do so. From this minimum a series of moderate budding years followed by a well-timed, maximum budding year are required for the flies to reach maximum abundance again, and it most frequently happens that some vicissitude, such as the non-fulfilment of correlation in time or space between flies and buds, prevents the flies from having maximum numbers in each year of maximum buds. Fortunately for the beekeeper who depends on a heavy crop of blossom for his honey harvest, it is the exception rather than the rule to get maximum galling coincident with maximum flower-bud production.

In some districts in which conditions are very favourable to *E. camaldulensis*, a good deal of bud formation takes place every year, with a certain biennial maximum budding, super-imposed on the annual. In such a district a good deal of galling is always present, with an occasional heavy maximum.

The biennial life cycle of some of the wasps so frequently associated with the flies in galling complicates the matter further. Many years of quantitative study would have to be made, however, before an authoritative statement about the relative importance of the rôles played by the various insects concerned in the galling could be arrived at. The availability of suitable breeding places is considered by the author to be the principal factor contributing to the big fluctuations in the numbers of flies from year to year in the flower-bud-galling species.

The flies using leaf bud, shoot tip, and other vegetative parts of the tree on which to form galls have never been seen to reach the same great abundance as the flower-bud-galling species, although they may often be common, as they are sometimes on the leaves of *E. Stuartiana*. The reason for this is probably twofold. In the first place, suitable young shoots or leaf-buds are subject to wide variations in abundance from year to year but not to the same extent as the flower buds. In the second place, parasites appear to play a bigger part in keeping the numbers

to a more even, steady density on the vegetative than in the reproductive galls. The part played by parasites may therefore be considered as a separate section.

Parasites and Other Insects in Relation to Abundance of Flies.

Some chalcid gall-formers have been bred out from the same galls in *E. macrorrhyncha* as the *Fergusonina* flies. These insects were not direct parasites but competitors within the galls, the effect of their inhabiting the same galls affecting the numbers of flies emerging. The gall wasps lay their eggs, mostly in buds already galled by the flies, and the larvae hatching from the wasp eggs produce separate gall chambers which have already been described in detail. These chambers have strong, hard walls which were impenetrable barriers to gall flies ready to emerge if they happened to be in their path. In addition to this, all the buds galled by the wasps became very woody so that the operculum was fused to the body of the gall, and consequently could not open to release the flies at the appointed time. Large numbers of flies were entombed in the galls by this means, and died there. The only chance they had of emerging was when a wasp, more advanced in its life history period than the others, cut a tunnel to the exterior through the wall of the gall, so that flies which happened to be mature at the time this hole was cut, and which were close to the hole, could squeeze through. The wasps have not been found in such great numbers on many trees, but on one tree observed, the branches were weighed down with galls and about 60% of the flies were unable to emerge owing to the presence of the wasps. The chief wasps concerned in this galling were *Epimegastigmus quinquesetae* Gir., *Ditropinotella compressiventris* Gir., *Eurytoma varirufipes* Gir., and an unidentified species of *Megastigmus*.

In addition to those chalcid wasps which lived in the same galls as the flies, many true parasites were bred out. One of the commonest chalcids was *Coelocyba eucalypti* Gir. Others have not been identified yet.

One of the larger insects bred out was a braconid wasp, the larva of which was found to feed indiscriminately on gall tissues and fly larvae. A full study of the hymenopterous fauna of the galls is reserved for a future occasion, so only the general observations can be recorded here.

It was found that in the bigger flower-bud galls the small chalcid parasites occurred mainly on larvae situated in the loculi near the outer surface of the bud. The larvae living deeper in the gall were free from most types of parasites, although they were preyed on by some predators, such as the braconid already mentioned. The parasites did not appear to be able to reach such a large percentage of the fly larvae in the flower-bud galls as in the leaf and stem-tip galls.

During 1934 a large number of galls were found on the leaves of *E. Stuartiana*. Parasites were common on them, so that from the galls about equal numbers of hymenopterous parasites and flies emerged. Galls were scarcer on trees of this species during 1935, in spite of the fact that much young growth appeared on the trees, growth which appeared suitable for galling. It was observed, however, that most of the flies emerged in 1934 during May, when there was little young growth available, so that a failure of co-ordination in time took place. Those galls which did appear in 1935 were examined, and in most of them nematodes were found with hymenopterous larvae or pupae. The empty skin of a larval fly of *Fergusonina* was found in each gall chamber, so it was clear that parasitism was very heavy.

From observation over a considerable period, and over a wide area, it would appear that parasites play an important rôle in controlling the steady density of

these leaf-galling species because, although they are liable to fluctuations, due to changes in the amount of available suitable vegetative parts in which the larvae can develop, those fluctuations do not bring them either to such large or to such small numbers as the flower-bud species.* They are able always to maintain a widespread rather low density and the continuous availability of hosts gives the parasites opportunity for a fairly constant measure of control.

Summary and Conclusions.

Many galls on leaves, stem tips, leaf buds and flower buds of *Eucalyptus* trees are caused by the combined action of nematodes and small flies of the Agromyzid genus *Fergusonina*. The flies and nematodes are invariably found together in the gall, and their relationship is described as a true symbiosis.

The association is clearly of long standing and probably originated in an accidental parasitism by the ancestors of the nematodes which had been plant-parasites, on the ancestors of the flies which were probably leaf-tunnellers.

The life histories of the flies and the nematodes have been worked out and their interdependence revealed.

The fly larvae, which are described for the first time, carry chitinous structures on the dorsum which are of great taxonomic value and phylogenetic interest.

The nematodes are found in the galls as free-living females and males. The first generation living in the galls is composed of parthenogenetic females, and there is an alternation of generations during which a generation of fertilized females is parasitic in the adult fly. The adult fly deposits the nematode larvae in buds with her own eggs.

The taxonomics and affinities of the nematodes are discussed and a new subgenus *Anguillulina* (*Fergusobia*) is erected to contain them.

Insect parasites of the flies are common and their significance in controlling the numbers of flies is discussed.

Large fluctuations in the number of gall flies occur from season to season. In the flower-bud gall-forming species the erratic bud formation of the *Eucalyptus* is considered to be the factor mainly responsible for the fluctuations. In the leaf and stem-tip gall-formers, which are nowhere so plentiful as the flower-bud types, although availability of suitable young growth controls the numbers to some extent, parasites are thought to effect a considerable measure of control.

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* For a full discussion of the subject of fluctuations in animal populations due to various causes reference should be made to Nicholson (1933).

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EXPLANATION OF PLATES VI-VII.

Plate vi.

Fig. 1.—Galled (left) and young ungalled (right) flower-buds of *E. macrorrhyncha*. × 0·4.

Fig. 2.—Branches of *E. macrorrhyncha* carrying many galls. × 0·06.

Fig. 3.—Normal flower-buds of *E. Blakelyi*. × 0·6.

Fig. 4.—Galled flower-buds of *E. Blakelyi*. × 0·09.

Fig. 5.—Leaf-galls on *E. Stuartiana* (centre and left); shoot-galls on *E. macrorrhyncha* (right). × 0·2.

Figs. 6-13.—Photomicrographs of galled flower-buds of *E. macrorrhyncha*.

Figs. 6-12: A, outer layer of operculum; B, inner layer of operculum; C, anther primordium; N, nematode larvae; L, larva of fly; 1 and 4, wasp gall cavities.

Fig. 13: A, inner layer of cells with dense protoplasm surrounding fly gall cavity; B, middle layer of cells in fly gall cavity; C, outer limiting layer of cells in fly gall cavity; L, fly larva; N, nematodes; H, thin-walled parenchyma in wasp gall cavity; W, outer woody layer of cells bounding wasp gall cavity. Figs. 6, 13, × 12; figs. 7-12, × 6.

6. Young flower bud in which eggs of fly and nematode larvae had been laid three days previously. 7. Flower bud with tissues proliferating. 8. A more advanced stage. At this stage the eggs of the fly hatch. 9. More advanced stage of galling. 10. Normal flower bud same age as galled bud in Fig. 9. 11. Cross-section of galled flower-bud with only one gall cavity containing nematodes. This section misses the fly larva which accompanied nematodes. 12. Cross-section of galled flower-bud showing many gall cavities containing fly larvae and nematodes, and two wasp gall cavities. 13. Cross-section showing contrast between vegetable tissues surrounding the fly larva and nematodes, and the wasp larva.

Plate vii.

F.O., ovary of fly; F.E., egg of fly; N, larval nematodes; E, egg of nematode; F, globule forming part of fat body of fly.

Fig. 1.—Adult female of *Fergusonina*. × 13.

Fig. 2.—Ovaries of fly dissected out showing nematode larvae protruding from torn oviduct. × 17.

Fig. 3.—Photomicrograph showing eggs of fly in ovary and a nematode larva between them. × 100.

Fig. 4.—Photomicrograph of elements from haemocoel of fly. × 100.

Figs. 5-12.—Photomicrographs of nematodes:

5. Free living female, × 125. 6. Male, × 100. 7. Parasitic female from body of fly larva which was about to pupate moulting into final instar, × 150. 8. Parasitic female growing rapidly; stylet in this stage still apparent, × 75. 9. Parasitic female nearly full grown; stylet has disappeared and gut almost completely atrophied. From puparium of fly, × 100. 10. Parasitic female at later stage than Fig. 9. From puparium of fly, × 50. 11. Parasitic female fully developed and most of her eggs already laid. From adult female fly *F. tillyardi*. × 35. 12. Fully developed female from body cavity of adult fly *F. carteri*. × 50.